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Patent Application
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1. Title of Invention:
A Disk Electrophoresis Measuring Method
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Specification

1. Title of the Invention
A Disk Electrophoresis Measuring Method
2. Scope of Patent Claims
A disk electrophoresis measuring method with the following features. It continuously records or measures the light absorbance, fluorescence, refractivity or radioactivity of the phoresis image while the chromatogram is developing and the electrophoresis is underway. Said measurements are taken either directly below or in direct proximity to the interface of the two-layer gel that has differing hydrogen ion concentrations and pore size.

3. Detailed Description of the Invention
This invention pertains to disk electrophoresis measuring methods and its purpose is to improve measuring precision, reduce measuring time and simplify the measuring process.

In order to assess the purity, analyze or separate proteins, nucleic acids, enzymes or blood serum, these samples are added to a gel carrier and an electric current is applied. The samples separate due to differences in their electrophoretic speeds, a phenomenon that has been known for a long time.

It is also known that if dextrin, polyacrylamide or other gels having molecular sieve characteristics are used as the gel in this phenomenon, the separation can be promoted even further.

Traditionally, disk electrophoresis methods that employ the pore size of these molecular sieves or the discontinuity of the hydrogen ion concentration, have been used to carry out separation processes like the one shown in Figure 1.

That is, a gel 2 with a large pore size (hereafter called a concentrating gel) is brought into contact with a gel 3 with a small pore size (hereafter called a dispersion gel) and an electric current is applied.

Then, for example, a sample 1, containing the component elements 4, 5 and 6 (the proteins, nucleic acids, enzymes or blood serum described above) would be added to the concentrating gel 2 as shown in Figure 1. The sample 1 would then pass phoretically through the concentrating gel 2 one after the other. Each of the separated components 4, 5 and 6 in the sample 1 would be concentrated in front of the interface 7 as in Figure 1 (b). They would undergo separation over time as shown in Figure 1 (c) and (d) before each component had become completely separated as shown in Figure 1 (e).

The light absorbance of each separated component would be measured and recorded, resulting in a

chromatogram. The pattern of said chromatogram would be used in the separation, analysis or the assessment of the purity of biomacromolecules in addition to the diagnoses of illnesses.

Traditionally, however, it has been necessary to undertake the following process in order to obtain said chromatogram.

After completing the electrophoresis, the gel would be removed and the protein, nucleic acid or other substance would be dyed and then decolorized using suitable methods and then, either photographs would be taken or the substance would be measured using a densitometer.

Alternately, a long gel could be inserted into the sample chamber of the densitometer and the gel moved at a fixed speed so that the changes in concentration over time could be measured and recorded.

These measurement methods have the following problems.

Firstly, it takes time for the sample to undergo complete separation by phoresis.

Secondly, it is generally extremely difficult to determine whether or not a colorless, transparent sample has separated completely and if the phoresis time goes on for too long, the components that have separated by phoresis will dissolve into the buffering solution tank.

Thirdly, gels that lack strength require handling operations such as the manual cutting into disks, dyeing, decolorization and other operations. This makes an experienced worker necessary.

Fourthly, the dispersion phenomenon becomes even more pronounced between the time that the electrophoretic separation is halted, the gel removed, dyed and decolorized, and the time that the measurements are made. This lowers and broadens the peak values of the chromatogram, degrading the resolution.

Fifthly, it takes time to obtain the chromatogram, with phoretic separation, dyeing, cutting into disks, measuring and recording.

Sixthly, since the manual processes cannot be eliminated, it would be impossible to automate devices for simultaneous measuring and recording of samples for mass screenings or other such large-scale applications.

Therefore, the purpose of this invention is to eliminate the above shortcomings, improve the precision of measurements, reduce measuring time and simplify the measurement process.

That is, this invention is a disk electrophoresis method that continuously measures and records the light absorbance and other characteristics of the electrophoresis image, immediately below or in the immediate vicinity of gel interfaces having differing pore sizes or hydrogen ion concentrations, at the same time that the electrophoresis is underway while the chromatogram is being developed. The following is a detailed description with reference to explanatory drawings.

In the explanatory drawing Figure 4, the sample 1, containing the components for separation 4, 5 and 6 is added to the tip of the concentrating gel 2. An electric current is then applied for the electrophoresis. The components for separation 4, 5 and 6 pass through the concentrating gel 2 and are concentrated at the interface 7 with the dispersion gel 3. Here, the order in which each of the components to be separated passes through this interface will differ due to their molecular weight, size, electrical charge or other characteristic and they will separate completely when they pass through as shown in Figure 4 (c). Sharp phoretic bands have already formed and pass through the dispersion gel 3 phoretically. As shown in Figures 4 (d) and (e), when each of the components to be separated passes through the interface 7, they each separate completely and pass through the dispersion gel 3 phoretically.

As previously explained in Figure 1, no suitable conventional method exists for measuring each of the components to be separated when they through the interface 7 and separate completely in the dispersion gel 3. However, when we observe and analyze the phenomenon of electrophoresis in detail, traditional research has clearly demonstrated that phoretic separation is complete by the time they pass through the interface 7.

Based on this scientific proof, one can conclude that making measurements immediately after the interface 7 has been passed through, will allow the realization of such advantages as improved measurement precision, reduced measuring time and a simplified measuring process. This is made even more clear by the explanatory drawing in Figure 5.

The light source 8, the detector 9 and the densitometer are positioned as close as possible to the interface 7 and so that the luminous flux passes through on the dispersion gel 3 side. Then, an electric current is applied and the chromatogram is developed while the electrophoresis is underway. The light absorbance of each of the components to be separated is measured continuously as they undergo phoresis in a straight line connecting the light source 8 and the detector 9, resulting in the chromatogram.

Additionally, when measuring and recording a large number of samples undergoing phoresis, many columns filled with gel could be positioned so that they were rotated sequentially into place where the light flux was and chromatograms could be recorded for each column in many colors or, conversely, if the column were fixed, the light flux and detector could be moved simultaneously to record the chromatograms in the same manner.

It is also clear that the irregular nature of the phoresis time of these samples can be easily varied by controlling the application of electric current or in the selection of the substances in the gel.

Therefore, as described above, based on a measuring method that automates the electrophoresis-measuring device, the measuring is started after the separation phoresis begins. Because no dyeing or decolorization operations are required, the measuring time can be reduced. Because the measurements are taken while the separation phoresis is underway, there is no dispersion phenomenon. It is clear that the many possible effects include improving the measuring precision by eliminating human influence on physiologically active substances as well as the simplification of the measuring process by automating the measuring device.

4. Brief Description of the Drawings

Among the explanatory drawings that describe this invention, Figure 1, Figure 2 and Figure 3 are explanatory drawings of conventional systems, while Figure 4 and Figure 5 show explanatory drawings of this invention.

1	Sample
2	Large Pore Size Gel (Concentrating Gel)
3	Small Pore Size Gel (Dispersion Gel)
4, 5, 6	Components to be Separated
7	Interface
8	Light Source
9	Detector
10	Recorder

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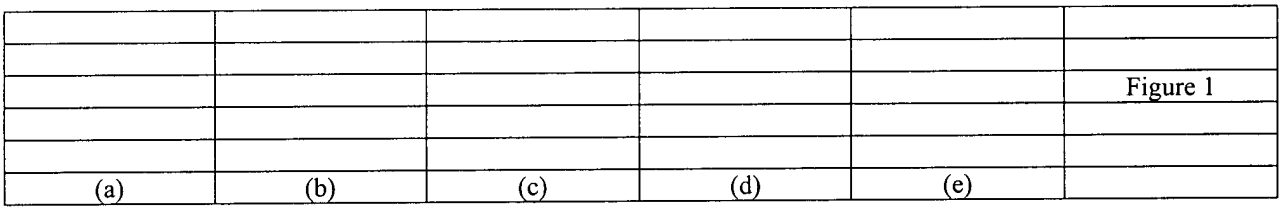

Figure 1

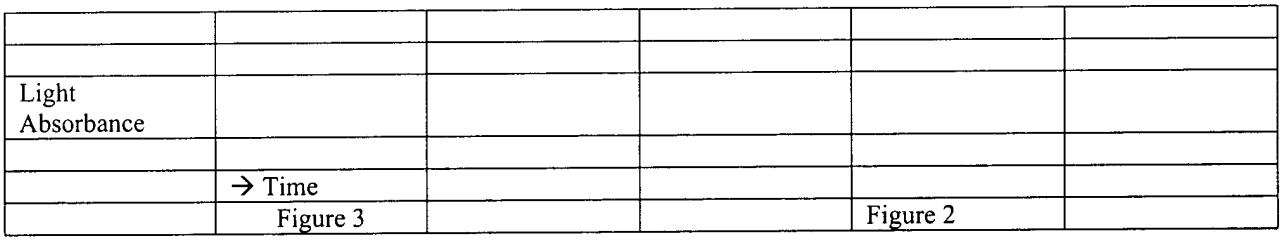

Figure 3

Figure 2

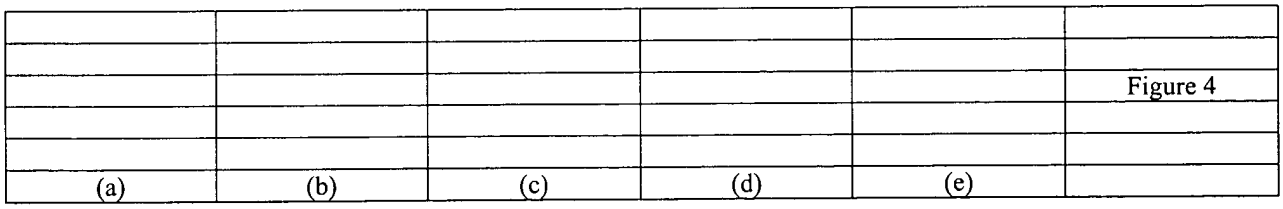

Figure 4

Figure 5